

# 利用苔蘚桿菌生產聚麩胺酸之搖瓶饋料批示培養探討

邱紫與、張耀南、洪淑嫻

E-mail: 9300219@mail.dyu.edu.tw

## 摘要

本研究目的在探討以苔蘚桿菌 *Bacillus licheniformis* CCRC 12826 為生產菌株，探討以液態搖瓶培養方式生產  $\gamma$ -聚麩胺酸之不同饋料基質組成濃度、饋料時間與饋料體積之研究。苔蘚桿菌培養於起始酸鹼值 pH 6.5 之 50 mL 修飾培養基中，於 37 溫度下，以 150 rpm 振盪培養 120h 時，可產生聚麩胺酸產量為 34.5g/L。當饋料基質組成分為 40 g/L 麩胺酸、42 g/L 檸檬酸、158 g/L 甘油、1 g/L 氯化銨，且在第 20h 培養時間添加 25 mL 於修飾培養基後繼續培養至 120h，可得到聚麩胺酸產量為 24.5 g/L；若將饋料添加體積由 25mL 濃縮為 5mL，即是饋料組成濃度為 200 g/L 麩胺酸、210 g/L 檸檬酸、790 g/L 甘油、5 g/L 氯化銨，如上述饋料與培養條件繼續培養 120h 後， $\gamma$ -聚麩胺酸產量即可達到 33.5 g/L，在 144h 培養後， $\gamma$ -聚麩胺酸產量卻能高達 45.3 g/L。雖然饋料程序使高產量  $\gamma$ -聚麩胺酸所需時間延長，但卻能提高產量，故饋料策略值得探討研究。

關鍵詞：苔蘚桿菌、 $\gamma$ -聚麩胺酸、饋料

## 目錄

第一章 緒論 .....	1	第二章 文獻回顧 .....	4	2.1 聚麩胺酸 (Poly glutamic acid) 之生合成 .....	4	2.2 苔蘚桿菌生產聚麩胺酸 .....	6	2.3 聚麩胺酸之應用 .....	13	2.3.1 聚麩胺酸在生物醫學材料 (Biomaterial) 的應用 .....	14	2.3.2 聚麩胺酸在抗癌藥物 (Antitumor drug) 的應用 .....	15	2.3.3 聚麩胺酸在環境工程領域的應用 .....	16	2.4 回應曲面法 .....	17	2.4.1 回應曲面法之原理 .....	18	2.4.2 二水準因子設計 (Two-Level Factorial Design) .....	20	第三章 材料與方法 .....	23	3.1 儀器設備 .....	23	3.2 實驗材料與方法 .....	23	3.2.1 材料 .....	23	3.2.2 培養方法 .....	24	3.3 回應曲面法之設計 .....	28	3.3.1 部分因子之實驗設計 .....	29	3.4 結果與討論 .....	31	第四章 結論與展望 .....	52	參考文獻 .....	54
--------------	---	----------------	---	--	---	----------------------	---	-------------------	----	---	----	--	----	----------------------------	----	-----------------	----	----------------------	----	--	----	-----------------	----	----------------	----	-------------------	----	----------------	----	------------------	----	--------------------	----	-----------------------	----	-----------------	----	-----------------	----	------------	----

## 參考文獻

1. 李昌憲、洪哲穎、熊光賓 (1992) 利用回應曲面法進行以 *Streptococcus faecalis* 生產酪胺酸脫氨酶之培養基最適化研究。中國農業化學會誌, 31:28-34。
2. 李琇鈴、周正俊、吳淳美 (1993) 利用回應曲面法尋求 *Streptococcus faecalis* 生產  $\gamma$ -decalactone 之最適條件。中國農業化學會誌, 31:28-34。
3. 范宜琮 (2001) 以苔蘚桿菌生產聚麩胺酸之研究。大葉大學環境工程學系碩士班碩士論文。
4. 洪哲穎 (1998) 回應曲面品質工程技術。工業局八十八年度人才培訓計畫研習班, 1998年11月, 私立義守大學, 高雄縣大樹鄉。
5. 洪哲穎、陳國誠 (1992) 回應曲面實驗設計法在微生物酵素生產上之應用, 39 (2):3-18。
6. 徐敬衡 (1994) 生物分解性微生物塑膠之開發。食品工業, 26(7):30-35。
7. 高馥君 (1992) 回應曲面在食品開發上的應用。食品工業, 24 (3):32 - 41。
8. Birrer, G. A., A. M. Cromwick, and R. A. Gross (1994)  $\gamma$ -Poly (glutamic acid) formation by *Bacillus licheniformis* 9945a: physiological and biochemical studies. *Int. J. Biol. Macromol.* 16:265-275
9. Bovarnick, M. (1942) The formation of extracellular D(-)-glutamic acid polypeptide by *Bacillus subtilis*. *J. Biol. Chem.* 145:415-424.
10. Box, G. E. P., and K. B. Wilson (1951) On the experimental attainment optimum conditions. *J. Roy. Stat. Soc. B13*:1-45.
11. Buchanan, R. L., and J. G. Philips (1990) Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. *J. Food Protect.*, 53:370-376
12. Cheng, C., Y. Asada, and T. Aida (1989) Production of  $\gamma$ -polyglutamic acid by *Bacillus subtilis* A35 under denitrifying conditions. *Agric. Biol. Chem.* 53:2369-2375.
13. Chun, L., D. F. Yu, A. Newman, F. Cabral, C. Stephens, N. Hunter, L. Milas, and S. Wallace (1998) Complete regression of well-established tumors using novel water-soluble poly (L-glutamic acid)-paclitaxel conjugate. *Cancer Res.* 58:2404-2409.
14. Chun, L., J. E. Price, L. Milas, N. R. Hunter, S. Ke, W. Tansey, C. Charnsagavej, and S. Wallace (1999) Antitumor activity of poly (L-glutamic acid)-paclitaxel on syngeneic and xenografted tumors. *Clin. Cancer Res.* 5:891-897.
15. Cromwick, A. M., and R. A. Gross (1995a) Effect of manganese ( ) on *Bacillus licheniformis* ATCC9945a physiology and  $\gamma$ -poly (glutamic acid) formation. *Int. J. Biol. Macromol.* 17:259-267.
16. Cromwick, A. M., and R. A. Gross (1995b) Investigation by NMR of metabolic routes to bacterial  $\gamma$ -poly (glutamic acid) using <sup>13</sup>C labeled citrate and glutamate as media carbon source. *Can. J. Microbiol.* 41:902-909.
17. Cromwick, A.

M., G. A. Birrer, and R. A. Gross (1996) Effects of pH and aeration on  $\gamma$ -poly (glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures. *Biotechnol. Bioeng.* 50:222-227. 18. Fujii, H. (1963) On the formation of mucilage by *Bacillus natto*. Part III Chemical constitutions of mucilage in natto (1). *Nippon Nogeikagaku Kaishi.* 37:407-411. 19. Goto, A., and M. Kunioka (1994) Biosynthesis of poly ( $\gamma$ -glutamic acid) from L-glutamic acid, citric acid, and ammonium sulfate in *Bacillus subtilis* IFO3335. *Biosci. Biotechnol. Biochem.* 56:1031-1035. 20. Haltrich, D., M. Press, and W. Steiner (1993) Optimization of a culture medium for increased xylanase production by a wild strain of *Schizophyllum commune*. *Enzyme Microb. Technol.* 15:854-860. 21. Ito, Y., T. Tanada, T. Ohmachi, and Y. Asada (1996) Glutamic acid independent production of poly ( $\gamma$ -glutamic acid) by *Bacillus subtilis* TAM-4. *Biosci. Biotechnol. Biochem.* 60:1239-1242. 22. Kautola, H., and Y. Y. Linko (1989) Fumaric acid production from xylose by immobilized *Rhizopus arrhizus* cells. *Appl. Microbiol. Biotechnol.* 31:448-452. 23. Ko, Y. H., and R. A. Gross (1998) Effects of glucose and glycerol on  $\gamma$ -poly (glutamic acid) formation by *Bacillus licheniformis* ATCC9945a. *Biotechnol. Bioeng.* 57:430-437. 24. Kubota, H., Matsunobu T. M., Uotani K., Takebe H., Satoh A., Tanaka T., and Taniguchi M. (1993) Production of poly( $\gamma$ -glutamic acid) by *Bacillus subtilis* F-2-01. *Biosci. Biotechnol. Biochem.* 57:1212-1213. 25. Leonard, C. G., R. D. Housewright, and C. B. Thorne (1958a) Effect of metal ions on glutamyl polypeptide synthesis by *Bacillus subtilis*. *J. Bacteriol.* 76:499-503. 26. Leonard, C. G., R. D. Housewright, and C. B. Thorne (1958b) Effect of metal ions on the optical specificity of glutamine synthetase and glutamyl transferase of *Bacillus licheniformis*. *Biochem. Biophys. Acta.* 62:432-434. 27. Maddox, I. S., and S. H. Richert (1977) Production of gibberellic acid using a dairy waste as the basal medium. *Appl. Environ. Microbiol.* 33:201-202. 28. Mudahar, S., R. T. Toledo, J. D. Floros, and J. J. Jen (1989) Optimization of carrot dehydration Process using response surface methodology. *J. Food Sci.*, 54:714-719. 29. Murao, S. (1969) On the polyglutamic acid fermentation. *Kobunshi.* 16:1204-1212. 30. Ontni, Y., Y. Tabata, and Y. Ikada (1996a) A new biological glue from gelatin and poly (L-glutamic acid). *J. Biomed. Mater. Res.* 31:157-166. 31. Ontni, Y., Y. Tabata, and Y. Ikada (1996b) Rapidly curable biological glue composed of gelatin and poly (L-glutamic acid). *Biomater.* 17:1381-1391. 32. Ontni, Y., Y. Tabata, and Y. Ikada (1998a) Effect of additives on gelation and tissue adhesion of gelation poly (L-glutamic acid). *Biomater.* 19:2167-2173. 33. Ontni, Y., Y. Tabata, and Y. Ikada (1998b) Hemostatic capability of rapidly curable from gelatin poly (L-glutamic acid) and carbodiimide. *Biomater.* 19:2091-2098. 34. Ontni, Y., Y. Tabata, and Y. Ikada (1999) Sealing effect of rapidly curable gelatin-poly(L-glutamic acid) hydrogel glue on lung air leak. *Ann. Thorac. Surg.* 67:922-926. 35. Perez-Camero, G., F. Congregado, J. J. Bou, and S. Munoz-Cuerra (1999) Biosynthesis and Ultrasonic degradation bacterial poly ( $\gamma$ -glutamic acid). *Biotechnol. Bioeng.* 63:110-115. 36. Prapulla, S. G., S. Jacob, N. Chand, D. Rajalakshmi, and N. G. Karanth (1992) Maximization of lipid production by *Rhodotroula gracilis* CFR-A using response surface methodology. *Biotechnol. Bioeng.* 40:965-969. 37. Sawamura, S. (1913) On *Bacillus natto*. *J. Coll. Agric.* 5:189-191. 38. Shih, I. L., and Y. T. Van (2001) The production of poly( $\gamma$ -glutamic acid) from microorganisms and its various applications. *Biores. Technol.* 79:207-225. 39. Thorne, C. B., C. G. Gomez, G. R. Blind, and R. D. Housewright (1953) Synthesis of glutamic acid and glutamyl polypeptide by *Bacillus anthracis*. Factors affecting peptide production in synthetic liquid media. *J. Bacteriol.* 65:472-478. 40. Thorne, C. B., C. G. Gomez, G. R. Blind, and R. D. Housewright (1954) Production of glutamyl polypeptide by *Bacillus subtilis*. *J. Bacteriol.* 65:307-315. 41. Troy, F. A. (1973) Chemistry and biosynthesis of the poly( $\gamma$ -D-glutamyl) capsule in *Bacillus licheniformis*. 1. Properties of the membrane-mediated biosynthetic reaction. *J. Biol. Chem.* 248:305-316. 42. Ward, R. M., R. F. Anderson, and F. K. Dean (1963) Polyglutamic acid production by *Bacillus subtilis* NRRL B-2612 grown on wheat gluten. *Biotechnol. Bioeng.* 5:41-48. 43. Yokoi, H., O. Natsuda, J. Hirose, S. Hayashi, and Y. Takasaki (1995) Characteristics of a biopolymer flocculant produced by *Bacillus* sp. PY-90. *J. Ferment. Bioeng.* 79:378-380. 44. Yokoi, H., T. Arima, J. Hirose, S. Hayashi, and Y. Takasaki (1996) Flocculation properties of poly( $\gamma$ -glutamic acid) produced by *Bacillus subtilis*. *J. Ferment. Bioeng.* 82:84-87. 45. Yoon, S. H., J. H. Do, S. Y. Lee, and H. N. Chang (2000) Production of poly- $\gamma$ -glutamic acid by fed-batch culture of *Bacillus licheniformis*. *Biotechnol. Lett.* 22:585-588.